CHAPTER 2

MATERIALS AND METHODS

1. Materials

1.1 Model drug - Indomethacin (Lot. No. 850602, China National-chemicals Imp. Exp. Corp., China.)

1.2 Additives - Methylcellulose (Premium grade, The Dow Chemical Company, U.S.A.)

: Methocel A 15LV (Lot. No. 83101221A)

: Methocel A 4C (Lot. No. 83051611A)

: Methocel A 4M (Lot. No. 83101602A)

- Hydroxypropylmethylcellulose (Premium grade, The Dow Chemical Company, U.S.A.)

: Methocel E 5 (Lot. No. 85103311E)

: Methocel E 15LV (Lot. No. 85101221E)

: Methocel K 4M (Lot NO. 85022101K)

: Methocel K 100M (Lot. NO. 85100501K)

- Hydroxypropylmethylcellulose phthalate (The Dow Chemical Company, U.S.A.)

: HPMCP HP-50 (Lot. NO. 23014)

- Ethylcellulose 10 cps. (The Dow Chemical Company, U.S.A.)
- Talcum
- Magnesium stearate

1.3 Dissolution media

- Dihydrogen potassium phosphate, AR grade (MERCK,Germany)
- Sodium hydroxide, AR grade (MERCK, Germany)

1.4 Solvent - Methanol, AR grade (MERCK, Germany)

- Ethanol, AR grade (MERCK, Germany)

- Acetone, AR grade (BDH Chemicals Ltd. Pool, England.)

2. Equipment

- Analytical balance (Satorius A200s, Germany).
- Harvard trip balance (Ohaus Scale Corporation, U.S.A.).
- Hot air oven (Memmert, type UL 80, Germany).
- Carver Laboratory Press (PERKIN-ELMER, model C, Fred & Carver Inc, U.S.A.)
- Single punch machine (Viuhang Enginerring, No. 74, Thailand).
- Oscillating granulator (Viuhany Enginerring, Thailand).
- pH meter (Pye Model 232, Pye Unicam Ltd., England).
- Hardness tester (Schleuniger, Model 2E/205, Dr. K. Schleuniger Co., Switzerland).
- Micrometer (Teclock Co., Japan).
- Disintegration apparatus (Hanson Research Corporation, Model QC-21, U.S.A.)
- Dissolution apparatus (Hanson Research Corporation, Model 500-230, U.S.A.)
- Spectrophotometer (Spectronic 2000, BAUSCH & LOMB, U.S.A.)

3. Preparation of Indomethacin Sustained Release Tablet

3.1 Formulation

The amount of ingredients used in each formulation are represented in Table 11.

Table 11 : Formulation of Indomethacin Sustained Release Tablet.

Ingredients	Amount per tablet (mg)
Indomethacin	100
Cellulose derivative	%*
Talcum	0.5
Magnesium stearate	0.5

* Percent of polymer used in each formulation was represented in Tables 12 and 13.

3.2 Preparation of drug-polymer granules

Indomethacin was dried at 60°C for 6 hours before passed through sieve No. 40 mesh. All types and grades of cellulose derivatives, except HPMCP HP-50 were also passed through a 40 mesh sieve before used.

3.2.1 For Methocel A, E and K grades.

Indomethacin and polymer were weighed and mixed in a plastic bag by geometric dilution method. Distilled water was sprayed onto the powder and the mixture was mixed in a motar until wet mass was obtained. The wet mass was screened through a 16 mesh sieve and dried at 45°C for 6 hours before dry screening through a 20 mesh sieve.

3.2.2 For Ethylcellulose

Ethanol was sprayed onto the powder mixture instead of distilled water. The solvent was freely evaporated for 15 minutes in an open air before drying in an oven.

3.2.3 For HPMCP

It was used in form of binding solution prepared by dissolving in a mixture of water : acetone = 2:3 The solvent was also evaparated before drying in an oven.

Table 12: Percent of Polymer used in Each Formulation

Formulation	% Polymer								
	MC			HPMC				EC	HPMCP
	A 15LV	A 4C	A 4M	E 5	E 15LV	K 4M	K 100M		
Blank	–	–	–	–	–	–	–	–	–
1	1.0	–	–	–	–	–	–	–	–
2	5.0	–	–	–	–	–	–	–	–
3	7.5	–	–	–	–	–	–	–	–
4	10.0	–	–	–	–	–	–	–	–
5	15.0	–	–	–	–	–	–	–	–
6	20.0	–	–	–	–	–	–	–	–
7	–	5.0	–	–	–	–	–	–	–
8	–	10.0	–	–	–	–	–	–	–
9	–	15.0	–	–	–	–	–	–	–
10	–	20.0	–	–	–	–	–	–	–
11	–	–	5.0	–	–	–	–	–	–
12	–	–	10.0	–	–	–	–	–	–
13	–	–	15.0	–	–	–	–	–	–
14	–	–	20.0	–	–	–	–	–	–
15	–	–	–	1.0	–	–	–	–	–
16	–	–	–	5.0	–	–	–	–	–
17	–	–	–	7.5	–	–	–	–	–
18	–	–	–	10.0	–	–	–	–	–
19	–	–	–	15.0	–	–	–	–	–
20	–	–	–	20.0	–	–	–	–	–
21	–	–	–	–	1.0	–	–	–	–
22	–	–	–	–	5.0	–	–	–	–
23	–	–	–	–	7.5	–	–	–	–
24	–	–	–	–	10.0	–	–	–	–
25	–	–	–	–	15.0	–	–	–	–
26	–	–	–	–	20.0	–	–	–	–
27	–	–	–	–	–	5.0	–	–	–
28	–	–	–	–	–	10.0	–	–	–
29	–	–	–	–	–	–	3.0	–	–
30	–	–	–	–	–	–	–	5.0	–
31	–	–	–	–	–	–	–	10.0	–
32	–	–	–	–	–	–	–	15.0	–
33	–	–	–	–	–	–	–	20.0	–
34	–	–	–	–	–	–	–	–	1.0
35	–	–	–	–	–	–	–	–	3.0
36	–	–	–	–	–	–	–	–	5.0
37	–	–	–	–	–	–	–	–	7.0

Table 13: Percent of Polymer used in Combined Formulation

Formulation	% Polymer								
	MC			HPMC				EC	HPMCP
	A 15LV	A 4C	A 4M	E 5	E 15LV	K 4M	K 100M		
Combination 1	-	-	-	10.0	-	-	-	-	1.0
Combination 2	-	-	-	10.0	-	-	-	5.0	-
Combination 3	-	-	-	-	7.5	-	-	-	1.0

3.2.4 Combined Formulations

Combination 1 and 3 : Indomethacin and hydrophilic cellulose were thoroughly mixed before incorporating 1% of HPMCP solution.

Combination 2 : Indomethacin was mixed with both Methocel E 5 and EC then ethanol : H_2O (1:1) was sprayed onto the mixture till wet mass occurred.

The mixture of which passed through a 16 mesh seive were placed in an open air to let organic solvent freely evaporate before drying process.

3.2.5 For Blank Preparation

Indomethacin was also granulated with distilled water and the tablets were prepared with the same procedure as in other preparations.

3.3 Tableting

The granules were mixed with talcum and magnesium stearate which were passed through a 40 mesh sieve before compressed at two compressional pressures (500 and 1,000 lb) by Carver Laboratory Press using a 6 mm diameter flat faced punch.

For the selected scale-up formula, the granules were obtained by using an oscillating granulator and compressed on a single punch machine using 6 mm diameter flat faced punch.

4. In Vitro Studies

4.1 Weight variation

Twenty indomethacin sustained release tablets were individually weighed using an analytical balance.

4.2 Thickness and hardness

Tablets were measured by using a micrometer and a hardness tester. Six tablets of each formulation were determined.

4.3 Disintegration

Six tablets of each formulation were evaluated using USP disintegration apparatus with disc. Water as the immersion fluid was maintained at 37 ± 2°C.

4.4 Dissolution Studies

Nine hundred milliliters of 1:4 phosphate buffer pH 7.2 :deaerated water were placed in a glass vessel specifed in the USP dissolution test, the medium was equilibrated to 37 ± 0.5°C. One tablet was placed in a dry basket, specified in the compendium, and immersed in the medium at the center of the vessel and at 20 mm above the bottom of the vessel. The apparatus was operated at the speed of 100 rpm. Four tablets of each formulation were evaluated.

Ten milliliters of specimen were withdrawn at the time interval of 15, 30, 45 minutes, 1, 2, 4, 6, 9 and 12 hours or until indomethacin was completely released. The same quantity of medium was added immediately after each sampling to keep the volume of the medium constant during the experiment.

Each sample was filtered through paper filter (Whatman No.1). The first two milliliters of filtrate were discharded. The absorbance of the filtrate was determined spectrophotometrically in a 1-cm cell at 318 nm.

The amount of indomethacin released at any time interval was calculated from the standard absorbance - concentration curve. A cumulative correction was made for the previously removed sample to determine the total amount of drug released.

5. Standard Curve of Indomethacin

Indomethacin 50.0 mg was accurately weighed and dissolved in 8 ml of methanol. The solution was then adjusted to 100.0 ml with phosphate buffer pH 7.2 and was used as stock solution.

The stock solution was individually pipetted, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 ml, into a 100.0 ml volumetric flask and diluted to volume with 1:4 phosphate buffer pH 7.2 : deaerated water. The final concentration of each solution was 10, 15, 20, 25, 30, 35 and 40 μg/ml, respectively.

The absorbance of known drug concentration was determined by a double beam spectrophotometer in a 1-cm cell at 318 nm. The 1:4 phosphate buffer pH 7.2: deaerated water was used as a blank solution. Each concentration was determined in duplicate.

Preparation of Phosphate Buffer pH 7.2 (28)

The amount of 34.7 ml of 0.2M NaOH and 50.0 ml of 0.2M KH_2PO_4 were mixed and diluted to 200.0 ml with deaerated water, and adjusted to pH 7.2 with 1 N NaOH.